NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 454

TOXICOLOGY AND CARCINOGENESIS STUDIES OF NICKEL SULFATE HEXAHYDRATE

(CAS NO. 10101-97-0)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge while supplies last from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). Listings of all published NTP reports and ongoing studies are also available from NTP Central Data Management. The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF NICKEL SULFATE HEXAHYDRATE

(CAS NO. 10101-97-0)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

July 1996

NTP TR 454

NIH Publication No. 96-3370

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and reported findings

J.K. Dunnick, Ph.D., Study Scientist
D.A. Bridge, B.S.
J.R. Bucher, Ph.D.
R.E. Chapin, Ph.D.
M.P. Dieter, Ph.D.
M.R. Elwell, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
J.R. Hailey, D.V.M.
J.K. Haseman, Ph.D.
A. Radovsky, D.V.M., Ph.D.
G.N. Rao, D.V.M., Ph.D.
G.S. Travlos, D.V.M.
D.B. Walters, Ph.D.
K.L. Witt, M.S., Oak Ridge Associated Universities

Experimental Pathology Laboratories, Inc.

Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
S. Botts, D.V.M., Ph.D.
E.T. Gaillard, D.V.M., Ph.D.

Dynamac Corporation

Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

Lovelace Inhalation Toxicology Research Institute

Conducted 16-day studies, evaluated pathology findings

R.O. McClellan, D.V.M., Director
C.H. Hobbs, D.V.M., Principal Investigator
J.M. Benson, Ph.D., Study Director
D.G. Burt, D.V.M.
R.L. Carpenter, Ph.D.
F.F. Hahn, D.V.M., Ph.D.
P.J. Haley, D.V.M.
J.A. Pickrell, D.V.M., Ph.D.

Conducted 13-week studies, evaluated pathology findings

R.O. McClellan, D.V.M., Director
C.H. Hobbs, D.V.M., Principal Investigator
J.M. Benson, Ph.D., Study Director
D.E. Bice, Ph.D.
D.G. Burt, D.V.M.
Y.S. Cheng, Ph.D.
F.F. Hahn, D.V.M., Ph.D.
J.A. Pickrell, D.V.M., Ph.D.
G.M. Shopp, Jr., Ph.D.

Conducted 2-year studies, evaluated pathology findings

C.H. Hobbs, D.V.M., Principal Investigator
J.M. Benson, Ph.D., Study Director
E.B. Barr, M.S.
D.G. Burt, D.V.M.
Y.S. Cheng, Ph.D.
G.L. Finch, Ph.D.
F.F. Hahn, D.V.M., Ph.D.
P.J. Haley, D.V.M., Ph.D.
K.R. Maples, Ph.D.
K.J. Nikula, D.V.M., Ph.D.

NTP Pathology Working Group

Evaluated slides, prepared pathology report on rats (2 June 1994)

M.P. Jokinen, D.V.M., Chair
Pathology Associates, Inc.
J. Cullen, V.M.D., Ph.D., Observer
NiPERA, Inc.
D. Dixon, D.V.M., Ph.D.
National Toxicology Program
E.T. Gaillard, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
J.R. Hailey, D.V.M.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
K.T. Morgan, B.V.Sc., Ph.D.
Chemical Industry Institute of Toxicology
A. Radovsky, D.V.M., Ph.D.
National Toxicology Program

Analytical Sciences, Inc.

Provided statistical analyses

R.W. Morris, M.S., Principal Investigator
N.G. Mintz, B.S.
S. Rosenblum, M.S.

Evaluated slides, prepared pathology report on mice (4 Month 1993)

R.A. Herbert, D.V.M., Ph.D., Chair
National Toxicology Program
S. Botts, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
D. Dixon, D.V.M., Ph.D.
National Toxicology Program
S.L. Eustis, D.V.M., Ph.D.
National Toxicology Program
J. Everitt, D.V.M.
Chemical Industry Institute of Toxicology
J.R. Hailey, D.V.M.
National Toxicology Program
J. Swenberg, D.V.M., Ph.D., Observer
NiPERA, Inc.
K. Takahashi, D.V.M., M.Sc., Ph.D.
National Toxicology Program

Biotechnical Services, Inc.

Prepared Technical Report

D.D. Lambright, Ph.D., Principal Investigator
C.M. Bailey, B.S. Pharm.
J.R. Carlton, B.A.
S.R. Gunnels, M.A.
T.A. King-Hunter, B.S.
S.M. Swift, B.S.

CONTENTS

ABSTRACT

$NiSO_4 \cdot 6H_2O$

NICKEL SULFATE HEXAHYDRATE

CAS No. 10101-97-0

Chemical Formula: $NiSO_4 \cdot 6H_2O$ Molecular Weight: 262.86

Synonyms: Blue salt; hexahydrate, nickel (2+) salt; nickel monosulfate hexahydrate; nickel (2+) sulfate hexahydrate; nickel (II) sulfate hexahydrate; nickel sulphate hexahydrate; nickelous sulfate hexahydrate; nickelous sulphate hexahydrate; single nickel salt, sulfuric acid

Nickel sulfate hexahydrate is used in nickel plating, as a mordant in dyeing and printing textiles, as a blackening agent for zinc and brass, and in the manufacture of organic nickel salts. Nickel sulfate hexahydrate was nominated by the National Cancer Institute to the NTP as part of a class study of nickel compounds for which there was little information on the toxic and carcinogenic effects of inhalation exposure. Male and female F344/N rats and $B6C3F_1$ mice were exposed to nickel sulfate hexahydrate (greater than 98% pure) by inhalation for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in L5178Y mouse lymphoma cells.

16-Day Study in Rats

Groups of five male and five female F344/N rats were exposed to 0, 3.5, 7, 15, 30, or 60 mg nickel sulfate hexahydrate/m^3 (equivalent to 0, 0.7, 1.4, 3.1, 6.1, or 12.2 mg nickel/m^3). Rats were exposed on weekdays only, for a total of 12 exposure days during a 16-day period. Additional groups of four or five male and female F344/N rats were exposed to 0, 3.5, 15, or 30 mg nickel sulfate hexahydrate/m^3 for tissue burden studies. In the core study, two 60 mg/m^3 males, one 30 mg/m^3 female, and all 60 mg/m^3 females died before the end of the study. Final mean body weights of all exposed groups of males and females were significantly lower than those of the controls, as were mean body weight gains of male rats. Clinical findings included increased rates of respiration and reduced activity levels in rats in all exposure groups, except those exposed to 3.5 mg/m^3. Absolute lung weights of 60 mg/m^3 males and of all exposed groups of females were significantly greater than those of the controls, as were the relative lung weights of all exposed groups of males and females. Inflammation (including degeneration and necrosis of the bronchiolar epithelium) occurred in the lungs of all exposed groups of males and females. Atrophy of the olfactory epithelium occurred in the nasal passages of all exposed groups of males (except 60 mg/m^3) and in 15, 30, and 60 mg/m^3 females. Lymphoid hyperplasia in the bronchial or mediastinal lymph nodes was observed in 30 mg/m^3 males and in 60 mg/m^3 males and females. The concentration of nickel in the lungs of all exposed groups of males and females was greater than in control animals.

16-Day Study in Mice

Groups of five male and five female $B6C3F_1$ mice were exposed to 0, 3.5, 7, 15, 30, or 60 mg nickel sulfate hexahydrate/m^3. Mice were exposed on weekdays only, for a total of 12 exposure days during a 16-day period. Additional groups of

five male and five female B6C3F$_1$ mice were exposed to 0 or 3.5 mg nickel sulfate hexahydrate/m^3 for tissue burden studies. All core study mice exposed to 7 mg/m^3 or greater died before the end of the study; all control and 3.5 mg/m^3 mice survived to the end of the study. Final mean body weights and weight gains of 7, 15, 30, and 60 mg/m^3 males and females were significantly less than those of the controls, and clinical findings in these groups included emaciation, lethargy, and rapid respiration rates. Absolute and relative lung weights of male and female mice exposed to 7 mg/m^3 or greater were significantly greater than those of the controls. Only tissues from mice exposed to 0, 3.5, or 7 mg/m^3 were examined histopathologically. Inflammation occurred in the lungs of 3.5 and 7 mg/m^3 males and females; necrosis of the alveolar and bronchiolar epithelium was a component of the inflammation in 7 mg/m^3 males and females. In addition, atrophy of the olfactory epithelium of the nasal passages was observed in 3.5 mg/m^3 males and females. Nickel concentrations in the lungs of mice exposed to 3.5 mg/m^3 were greater than those in the controls.

13-Week Study in Rats

Groups of ten male and ten female F344/N rats were exposed to 0, 0.12, 0.25, 0.5, 1, or 2 mg nickel sulfate hexahydrate (equivalent to 0, 0.03, 0.06, 0.11, 0.22, or 0.44 mg nickel/m^3), 5 days per week for 13 weeks. Additional groups of six male and six female F344/N rats were exposed to 0, 0.12, 0.5, or 2 mg nickel sulfate hexahydrate/m^3 for tissue burden studies. In the core study, one 2 mg/m^3 male rat died before the end of the study; all other males and all females survived until the end of the study. Final mean body weights and body weight gains of all exposed groups were similar to those of the controls. There were no significant clinical findings noted during the study. Exposure-related increases in neutrophil and lymphocyte numbers occurred and were most pronounced in female rats. With the exception of 0.12 mg/m^3 rats, absolute and relative lung weights of all exposed groups were generally significantly greater than those of the controls. Exposure-related increases in the incidence and severity of inflammatory lesions (alveolar macrophages, chronic inflammation, and interstitial infiltration) occurred in the lungs of all exposed groups of males and females. Lymphoid hyperplasia of the bronchial and/or mediastinal lymph nodes occurred in males exposed to 0.5 mg/m^3 or greater. Atrophy of the olfactory epithelium occurred in males and females exposed to 0.5, 1, and 2 mg/m^3 and in 0.25 mg/m^3 females. The concentration of nickel in the lungs of 0.5 and 2 mg/m^3 rats was greater than that in the lungs of control animals at 4, 9, and 13 weeks for males and at 13 weeks for females.

13-Week Study in Mice

Groups of ten male and ten female B6C3F$_1$ mice were exposed to 0, 0.12, 0.25, 0.5, 1, or 2 mg nickel sulfate hexahydrate, 5 days per week for 13 weeks. Additional groups of up to five or six male and female B6C3F$_1$ mice were exposed to 0, 0.12, 0.5, or 2 mg nickel sulfate hexahydrate/m^3 for tissue burden studies. In the core study, four control males, three control females, and one 0.12 mg/m^3 male died before the end of the study; the deaths were not considered to be chemical related, and all other mice survived to the end of the study. The final mean body weights and body weight gains of all exposed groups were similar to those of the controls. There were no chemical-related clinical findings. Hematology changes similar to those reported in female rats occurred in female mice, but the mice were minimally affected. The absolute and relative lung weights of 1 mg/m^3 males and 2 mg/m^3 males and females were significantly greater than those of the controls. Increased numbers of alveolar macrophages occurred in all males and females exposed to 0.5 mg/m^3 or greater. Chronic active inflammation and fibrosis occurred in 1 and 2 mg/m^3 males and females. Lymphoid hyperplasia of the bronchial lymph node and atrophy of the olfactory epithelium in the nasal passages were observed in 2 mg/m^3 males and females. Nickel concentration in the lung of 2 mg/m^3 females was significantly greater than in control animals.

2-YEAR STUDY IN RATS

Groups of 63 to 65 male and 63 to 64 female rats were exposed to nickel sulfate hexahydrate by inhalation at concentrations of 0, 0.12, 0.25, or 0.5 mg/m^3 (equivalent to 0, 0.03, 0.06, or 0.11 mg nickel/m^3). Animals were exposed for 6 hours plus T_{90} (8 minutes) 5 days per week for 104 weeks. Five male and five female rats from each group were evaluated at 7 months for histopathology; an additional seven males and seven females from each group were evaluated at 7 months for nickel tissue burden in the lung and kidney; and five males and five females from each group were evaluated at 15 months for alterations in hematology, nickel tissue burden in the lung and kidney, and histopathology.

Survival, Body Weights, Clinical Findings, and Hematology

The survival rates of all exposed groups of males and females were similar to those of the controls. Mean body weights of 0.5 mg/m^3 female rats were slightly lower (6% to 9%) than those of the controls throughout the second year of the study; final mean body weights of all exposed groups of males and 0.12 and 0.25 mg/m^3 females were similar to those of the controls. There were no clinical findings or hematology differences that were considered to be related to nickel sulfate hexahydrate administration.

Pathology Findings

No exposure-related neoplasms occurred in male or female rats exposed by inhalation to nickel sulfate hexahydrate for 2 years. Increased incidences of inflammatory lung lesions were generally observed in all exposed groups of male and female rats at the end of the study. The incidences of chronic active inflammation, macrophage hyperplasia, alveolar proteinosis, and fibrosis were markedly increased in male and female rats exposed to 0.25 or 0.5 mg/m^3. Increased incidences of lymphoid hyperplasia in the bronchial lymph nodes occurred in 0.5 mg/m^3 male and female rats at the end of the 2-year study. The incidences of atrophy of the olfactory epithelium in 0.5 mg/m^3 males and females were significantly greater than those in controls at the end of the study.

Tissue Burden Analyses

Lung nickel burdens in exposed male and female rats were greater than those in the controls at the 7- and 15-month interim evaluations, and lung nickel burdens values increased with increasing exposure concentration.

2-YEAR STUDY IN MICE

Groups of 80 male and 80 female mice were exposed to nickel sulfate hexahydrate by inhalation at concentrations of 0, 0.25, 0.5, or 1 mg/m^3 (equivalent to 0, 0.06, 0.11, or 0.22 mg nickel/m^3). Animals were exposed for 6 hours plus T_{90} (8 minutes) 5 days per week for 104 weeks. Five male and five female mice from each group were evaluated at 7 months for histopathology; five males and five females from each group were evaluated at 7 months for nickel tissue burden in the lung and kidney; five males and five females from each group were evaluated at 15 months for alterations in hematology and histopathology; and five males and five females from each group were evaluated at 15 months for nickel tissue burden in the lung and kidney.

Survival, Body Weights, Clinical Findings, and Hematology

The survival rates of all exposed groups of males and females were similar to those of the controls. The mean body weights of 1 mg/m^3 males and of all exposed groups of females were lower than those of the controls during the second year of the study. There were no clinical findings or hematology differences considered to be related to chemical exposure.

Pathology Findings

Inflammatory lesions of the lung generally occurred in all exposed groups of male and female mice at the end of the 2-year study. These lesions included macrophage hyperplasia, chronic active inflammation, bronchialization (alveolar epithelial hyperplasia), alveolar proteinosis, and infiltrating cells in the interstitium. Incidences of macrophage hyperplasia and/or lymphoid hyperplasia occurred in the bronchial lymph nodes of most of the 1 mg/m^3 males and females and in some 0.5 mg/m^3 females at the end of the 2-year study. Atrophy of the olfactory epithelium was observed in 0.5 and 1 mg/m^3 males and in all exposed groups of females at the end of the 2-year study.

Tissue Burden Analyses

At the 7- and 15-month interim evaluations, lung nickel burden parameters measured in control and exposed groups were below the limit of detection. Absolute lung weights of 0.5 and 1 mg/m^3 lung burden study females were significantly greater than those of the controls at 15 months.

GENETIC TOXICOLOGY

Nickel sulfate hexahydrate (500 to 800 μg/mL) was tested for induction of trifluorothymidine resistance in L5178Y mouse lymphoma cells. A positive response was observed in the absence of S9. The test was not performed with S9.

CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity** of nickel sulfate hexahydrate in male or female F344/N rats exposed to 0.12, 0.25, or 0.5 mg/m^3 (0.03, 0.06, or 0.11 mg nickel/m^3). There was *no evidence of carcinogenic activity* of nickel sulfate hexahydrate in male or female B6C3F$_1$ mice exposed to 0.25, 0.5, or 1 mg/m^3 (0.06, 0.11, or 0.22 mg nickel/m^3).

Exposure of rats to nickel sulfate hexahydrate by inhalation for 2 years resulted in increased incidences of chronic active inflammation, macrophage hyperplasia, alveolar proteinosis, and fibrosis of the lung; lymphoid hyperplasia of the bronchial lymph node; and atrophy of the olfactory epithelium. Exposure of mice to nickel sulfate hexahydrate by inhalation for 2 years resulted in increased incidences of chronic active inflammation, bronchialization (alveolar epithelial hyperplasia), macrophage hyperplasia, interstitial infiltration, and alveolar proteinosis of the lung; lymphoid and macrophage hyperplasia of the bronchial lymph node; and atrophy of the olfactory epithelium.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 10. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Nickel Sulfate Hexahydrate

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Exposure concentrations	0, 0.12, 0.25, or 0.5 mg/m^3 (equivalent to 0, 0.03, 0.06, or 0.11 mg nickel/m^3)	0, 0.12, 0.25, or 0.5 mg/m^3 (equivalent to 0, 0.03, 0.06, or 0.11 mg nickel/m^3)	0, 0.25, 0.5, or 1 mg/m^3 (equivalent to 0, 0.06, 0.11, or 0.22 mg nickel/m^3)	0, 0.25, 0.5, or 1 mg/m^3 (equivalent to 0, 0.06, 0.11, or 0.22 mg nickel/m^3)
Body weights	Exposed groups similar to controls	0.5 mg/m^3 group lower than controls	1 mg/m^3 group lower than controls	Exposed groups lower than controls
2-Year survival rates	16/54, 16/53, 18/53, 21/53	22/53, 17/53, 28/54, 29/55	26/61, 23/61, 24/62, 25/62	34/61, 39/60, 45/60, 37/60
Nonneoplastic effects	Lung: chronic active inflammation (14/54, 11/53, 42/53, 46/53); macrophage hyperplasia (7/54, 9/53, 35/53, 48/53); alveolar proteinosis (0/54, 0/53, 12/53, 41/53); fibrosis (3/54, 6/53, 35/53, 43/53) Bronchial lymph node: lymphoid hyperplasia (0/51, 0/49, 3/47, 10/52) Nose (olfactory epithelium): atrophy (0/54, 0/52, 3/53, 7/53)	Lung: chronic active inflammation (14/52, 13/53, 49/53, 52/54); macrophage hyperplasia (9/52, 10/53, 32/53, 45/54); alveolar proteinosis (1/52, 0/53, 22/53, 49/54); fibrosis (8/52, 7/53, 45/53, 49/54) Bronchial lymph node: lymphoid hyperplasia (2/50, 1/52, 0/51, 11/49) Nose (olfactory epithelium): atrophy (0/51, 1/52, 1/53, 7/54)	Lung: chronic active inflammation (1/61, 2/61, 8/62, 29/61); bronchialization (1/61, 4/61, 19/62, 39/61); macrophage hyperplasia (6/61, 9/61, 35/62, 59/61); interstitial infiltration (1/61, 0/61, 3/62, 17/61); alveolar proteinosis (0/61, 0/61, 0/62, 42/61) Bronchial lymph node: lymphoid hyperplasia (2/46, 4/49, 2/45, 17/54); macrophage hyperplasia (0/46, 0/49, 8/45, 39/54) Nose (olfactory epithelium): atrophy (0/61, 0/61, 12/61, 37/60)	Lung: chronic active inflammation (1/61, 7/60, 14/60, 40/60); bronchialization (0/61, 9/60, 32/60, 45/60); macrophage hyperplasia (7/61, 24/60, 53/60, 59/60); interstitial infiltration (0/61, 4/60, 16/60, 39/60); alveolar proteinosis (0/61, 0/60, 11/60, 45/60) Bronchial lymph node: lymphoid hyperplasia (15/50, 9/54, 16/58, 26/56); macrophage hyperplasia (2/50, 0/54, 14/58, 37/56) Nose (olfactory epithelium): atrophy (3/61, 2/59, 1/60, 17/60)
Neoplastic effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology				
L5178Y mouse lymphoma cells gene mutations:	Positive without S9			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on nickel sulfate hexahydrate on November 29, 1994, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

Arnold L. Brown, M.D., Chair
University of Wisconsin Medical School
Madison, WI

Thomas L. Goldsworthy, Ph.D., Principal Reviewer
Department of Environmental Pathology and Toxicology
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

Meryl H. Karol, Ph.D.*
Department of Environmental Occupational Health
University of Pittsburgh
Pittsburgh, PA

Curtis D. Klaassen, Ph.D.
Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

Claudia S. Miller, M.D.
University of Texas Health Sciences Center
San Antonio, TX

Janardan K. Reddy, M.D.*
Department of Pathology
Northwestern University Medical School
Chicago, IL

Irma Russo, M.D., Principal Reviewer
Fox Chase Cancer Center
Philadelphia, PA

Louise Ryan, Ph.D.
Division of Biostatistics
Harvard School of Public Health and
Dana-Farber Cancer Institute
Boston, MA

Robert E. Taylor, M.D., Ph.D., Principal Reviewer
Department of Pharmacology
Howard University College of Medicine
Washington, DC

Mary Jo Vodicnik, Ph.D.*
Lilly MSG Development Center
Belgium

Jerrold M. Ward, D.V.M., Ph.D.
National Cancer Institute
Frederick, MD

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On November 29, 1994, the draft Technical Report on the toxicology and carcinogenesis studies of nickel sulfate hexahydrate received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of nickel sulfate hexahydrate by discussing the uses of the chemical and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplasms and nonneoplastic lesions in male and female rats and mice. The proposed conclusions were *no evidence of carcinogenic activity* in male and female F344/N rats and *no evidence of carcinogenic activity* in male and female B6C3F$_1$ mice.

Dr. Taylor, a principal reviewer, agreed with the proposed conclusions. He said that the exposure concentrations could have been slightly higher.

Dr. Goldsworthy, the second principal reviewer, agreed with the proposed conclusions although he also thought the exposure concentrations selected might have been higher, especially in mice. Dr. Goldsworthy observed that exposure concentrations selected for minimal to mild responses at 13 weeks resulted in minimal to mild changes at 2 years, including no target tissue weight changes in some circumstances. Dr. M.R. Elwell, NIEHS, commented that the high dose was based on the morphologic appearance of the lungs being similar to that in high dose animals in the nickel oxide study. Based on body weight decreases, he believed that a higher exposure concentration might have resulted in exceeding the maximum tolerated dose. Dr. Goldsworthy said that target tissue (lung) nickel concentrations were not observed at any exposure concentration in mice, and thus, exposure-response linkages could not be made, limiting extrapolation of data and comparison to other nickel studies. Dr. J.R. Bucher, NIEHS, explained that lung burden information would have been used if in the prechronic studies there had been a non-linear increase, i.e., an overload condition was reached with a particular dose. This did not occur, so in this case all exposure concentrations were selected based on inflammatory changes in the lung and decreases in body weight gain.

Dr. Russo, the third principal reviewer, agreed with the proposed conclusions. She stated that the lymph node hyperplasia should be documented in order to prove that the lesions represented a reactive process, either a reactive hyperplasia or a granulomatous reaction, versus monoclonal proliferation or early lymphoma.

Dr. Klaassen expressed surprise in view of the epidemiological data that the nickel compounds did not provide stronger evidence of carcinogenic activity in the NTP animal studies by the inhalation route. Dr. Dunnick noted the evidence for multiple exposures in the workplace and speculated that this could result in concurrent biologic events that might enhance cancer development. Dr. Goldsworthy commented again that since toxicity did not predict or relate to carcinogenicity, future studies with metals and inhaled toxicants should be more concerned with pulmonary function. Dr. G.W. Lucier, NIEHS, said that the discussions regarding dose selection and how one compares studies across a class of chemicals illustrate why the NTP in its more recent study designs is incorporating mechanistic markers or toxicokinetic profiles to enable better comparisons across organs and species.

Ms. D. Sivulka, executive director of the Nickel Producers Environmental Research Association, Inc. (NiPERA), commented on the discussion of evidence for nickel toxicity and carcinogenesis in humans and the presentation of the significance of findings relative to existing threshold limit values (TLVs). Ms. Sivulka said that because conclusions in the report were based on existing TLVs, an implication could be made that current regulations are not

protective of workers exposed to nickel compounds. Ms. Sivulka discussed the cohorts of workers exposed to nickel compounds that have been examined, and she said that the information obtained from these examinations shows no evidence of nickel-related increases in the incidence of nonneoplastic lesions in workers exposed to low nickel levels.

Dr. Taylor moved that the Technical Report on nickel sulfate hexahydrate be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, *no evidence of carcinogenic activity*. Dr. Klaassen seconded the motion, which was accepted unanimously with seven votes.